English translation of DE 19903713.2

Medicament to inhibit the expression of a given gene

The invention concerns a medicament and a use of double-stranded oligoribonucleotides.

DE 196 31 919 C2 describes an antisense RNA with particular secondary structures, whereby the antisense RNA is present in the form of a vector that codes for it. This antisense RNA concerns an RNA molecule that is complementary to regions of the mRNA. Inhibition of gene expression is effected by binding to these regions. This inhibition can be used, in particular, for the diagnosis and/or treatment of diseases, such as tumor diseases or viral infections. Unfortunately, the antisense RNA must be introduced into the cell in a quantity that is at least as great as the quantity of mRNA. The effectiveness of the known antisense method is not particularly high.

A medicament is known from US 5,712,257 that contains the mispaired double-stranded RNA (dsRNA) and biologically active mispaired fragments of dsRNA in the form of a ternary complex having an agent that is surface-active. The dsRNA used for this consists of synthetically produced single nucleic acid strands without a defined base sequence. The single strands combine into chance base pairings such that mispaired double strands are formed. The known dsRNA is used to inhibit the reproduction of retroviruses such as HIV. The retrovirus genome consists of double-stranded RNA that binds various proteins during reproduction of the retrovirus. The binding of these proteins, and thus the reproduction of the virus,

can be inhibited if non-specific dsRNA is introduced in high concentrations into the infected cells. This leads to competition between the non-specific dsRNA and the double-stranded viral RNA. The inhibitory effect or effectiveness of this method is small.

It is known from Fire, A. et al., NATURE, vol. 391, page 806, that dsRNA, one of whose strands is segmentally complementary to a nematode gene that is to be inhibited, inhibits expression of this gene very effectively. It is argued that the particular effectiveness of the dsRNA used in the cells of the nematode is not based on the antisense principle, but quite possibly on the catalytic characteristics of the dsRNA. This article makes no claims regarding the effectiveness of specific dsRNA in relation to the inhibition of gene expression, in human cells in particular.

The task of the present invention is to remove the shortcomings in accordance with the state of the art. In particular, an effective medicament, and an effective use for the manufacture of a medicament is to be disclosed, with which the inhibition of the expression of a given gene is effected.

This task is solved by the elements in Claims 1, 2, 13, and 14. Advantageous enhancements result from Claims 3 to 12 and 15 to 26.

In accordance with the provisions of the invention, a medicament is anticipated that contains at least one double-stranded oligoribonucleotide (dsRNA) to inhibit the expression of a given gene, whereby one strand of the dsRNA is at least

segmentally complementary to this gene. Surprisingly, it has been shown that dsRNA is suitable as a medicament to inhibit the expression of a given gene in human cells. Inhibition takes place at a concentration that is at least one order of magnitude lower than occurs using single-stranded oligoribonucleotides. The medicament that is the subject of this invention is highly effective. Lesser negative side effects are to be expected.

In accordance with further provisions of the invention, a medicament is anticipated that contains at least one vector for coding double-stranded oligoribonucleotides (dsRNA) to inhibit the expression of a given gene, whereby one strand of the dsRNA is at least segmentally complementary to this gene. The proposed medicament exhibits the above-mentioned advantages. The use of a vector can save on production costs, in particular.

Insofar as dsRNA is used as the active agent, it has been shown to be advantageous for the dsRNA to be present packaged in micellar structures, preferably in liposomes. The dsRNA can also be enclosed in natural viral capsids or capsids that have been synthesized by chemical or enzymatic means, or in structures that have been derived from them. The above-named characteristics make it possible to channel the dsRNA into the given target cells.

In accordance with a further enhancement feature, the dsRNA exhibits 10 to 1000, preferably 252 350, base pairs. Such dsRNA, or vectors that are anticipated to code same, can be synthetically or enzymatically produced using current methods.

The gene to be inhibited, preferentially an oncogene, can be expressible in eukaryotic cells or in pathogenic organisms, preferably in plasmodia. It can be a component of a virus or viroid that is preferably pathogenic in humans. The proposed medicament permits the treatment of genetically driven diseases, such as cancer and viral diseases.

The virus or viroid can also be one that is pathogenic in animals or plants. In this case, the medicament that is the subject of this invention permits the treatment of animal or plant diseases.

In accordance with a further enhancement feature, the dsRNA is at least segmentally double-stranded. Their ends can be modified to prevent degradation in the cell. This can decrease the potential for enzymatic attack.

In accordance with a further provision of the invention, the use of double-stranded oligoribonucleotides to produce a medicament to inhibit the expression of a given gene is anticipated, whereby one strand of the dsRNA is at least segmentally complementary to this gene. Surprisingly, such dsRNA is suitable for the production of a medicament to inhibit the expression of a given gene. In one use of dsRNA, inhibition occurs at concentrations that are one order of magnitude less than is the case when using single-stranded oligoribonucleotides. The use anticipated in this invention thus makes possible the production of particularly effective medicaments.

In accordance with a further provision of the invention, a use of a vector for coding double-stranded oligoribonucleotides

(dsRNA) is anticipated for the production of a medicament to inhibit the expression of the given gene, whereby one strand of the dsRNA is at least segmentally complementary to this gene. The use of a vector makes possible the production of particularly inexpensive and effective medicaments.

With regard to the use enhancements, see the description of the aforementioned elements.

Example:

An apparent single strand of RNA has been enzymatically synthesized from the only sequence protocol, using established methods.

Furthermore, the single strand of RNA that is complementary to it has also been synthesized. Subsequently, the single strand and the complementary single strand are fused into a dsRNA. The dsRNA created in this way contains a segment of the "immediate early gene" of the cytomegalovirus..

Experimental protocol:

A plasmid vector was constructed, the use of which allowed the necessary dsRNA to be produced. A polymerase chain reaction (PCR) to amplify the 363 base pairs of the 5'-end of the "immediate early gene" of the cytomegalovirus was used to construct this T7/SP6-transcription plasmid. Commercially available "positive control DNA" of the cytomegalovirus from the HeLaScribe Nuclear Extract *in vitro* transcription kit (Promega Co.) was used as the matrix. Oligodeoxyribonucleotides, whose sequence is identical or complementary to the ends of the above-mentioned region of the

"immediate early genes," served as the primer. The pGEM®-T vector (Promega Co.) was used as the cloning vector for the PCR product that was obtained. Transformation of E. coli XL1-blue ensued. The DNA plasmid of a selected clone, whose sequence had been checked by partial sequencing, was linearized with NcoI and SalI, respectively, and used as matrices for *in vitro* transcription with SP6-and T7-RNA polymerase, respectively (RiboMAX™ in vitro transcription kit, Promega).

The oligoribonucleotides obtained were purified and mixed in a sodium phosphate buffer (pH 6.5) in equimolar quantities in the presence of 100mM NaCl. After heating for a short time to 95°C, the mixture was slowly cold down over approximately 2.5 hours, such that formation of dsRNA was achieved by pairing both complementary single strands.

Test system with human nucleus extract:

Transcription efficiency of the given region of the "immediate early gene" of the cytomegalovirus in the presence of both single-stranded oligoribonucleotides and of dsRNA was determined using the HeLaScribe® Nuclear Extract *in vitro* transcription kit (Promega). This was done using the radioactivity incorporated in the "run off" transcripts of the [α^{32}P] ATP which is used as the substrate,. Separation of free ATP from the resulting transcription was done using gel electrophoresis. Evaluation of the gel was done with the help of a radioactivity detector (Instant Imager).

Results and conclusions:

A clear decrease in the quantity of transcription in the presence of dsRNA was seen in comparison with the control assay without RNA, as well as with the assays with single-stranded RNA. The effectiveness of dsRNA was achieved using small quantities, in particular less than 10% of that required by antisense technology to inhibit the translation of the required RNA concentration. The inhibitory effect of single-stranded antisense RNA would not have been detectable using this test system, because in this case inhibition takes place at the level of translation. Transcription was investigated here. The reduction in transcription quantity of a gene in the presence of dsRNA, which was observed in humans here for the first time, clearly demonstrates inhibition of the expression of the corresponding gene. This effect is attributed to a new type of mechanism conditioned by dsRNA.

Patent Claims

1. Medicament containing at least one double-stranded oligoribonucleotide (dsRNA) to inhibit the expression of a given gene, whereby one strand of the dsRNA is at least segmentally complementary to this gene.

2. Medicament containing at least one vector to code double-stranded oligoribonucleotides (dsRNA) to inhibit the expression of a given gene, whereby one strand of the dsRNA is at least segmentally complementary to this gene.

3. Medicament in accordance with Claim 1, whereby the dsRNA is present packaged in micellar structures, preferably in liposomes.

4. Medicament in accordance with Claim 1, whereby the dsRNA is enclosed in natural viral capsids or capsids that have been synthesized by chemical or enzymatic means, or in structures that have been derived from them.

5. Medicament in accordance with one of the previous claims, whereby the dsRNA exhibits 10 to 1000, preferably 250 to 350, base pairs.

6. Medicament in accordance with one of the previous claims, whereby the gene to be inhibited, preferably an oncogene, is expressible in eukaryotic cells.

7. Medicament in accordance with one of the previous claims, whereby the gene to be inhibited is expressible in pathogenic organisms, preferably in plasmodia.

8. Medicament in accordance with one of the previous claims, whereby the gene to be inhibited is a component of a virus or viroid.

9. Medicament in accordance with Claim 8, whereby the virus is a human pathogenic virus or viroid.

10. Medicament in accordance with Claim 8, whereby the virus or viroid is one that is pathogenic in animals or plants.

11. Medicament in accordance with one of the previous claims, whereby the dsRNA is segmentally double-stranded.

12. Medicament in accordance with one of the previous claims, whereby the ends of the dsRNA are modified to prevent degradation in the cell.

13. Use of a double-stranded oligoribonucleotide (dsRNA) to produce a medicament that inhibits the expression of a given gene, whereby one strand of the dsRNA is at least segmentally complementary to this gene.

14. Use of a vector to code double-stranded oligoribonucleotides (dsRNA) to produce a medicament that inhibits the expression of a given gene, whereby one strand of the dsRNA is at least segmentally complementary to this gene.

15. Use in accordance with Claim 13, whereby the dsRNA is present packaged in micellar structures, preferably in liposomes.

16. Use in accordance with Claim 13, whereby the dsRNA is enclosed in natural viral capsids or capsids that have been synthesized by chemical or enzymatic means, or in structures that have been derived from them.

17. Use in accordance with one of the Claims 13 to 16, whereby the dsRNA exhibits 10 to 1000, preferably 250 to 350, base pairs.

18. Use in accordance with one of the Claims 13 to 17, whereby the gene to be inhibited, preferably an oncogene, is expressible in eukaryotic cells.

19. Use in accordance with one of the Claims 13 to 18, whereby the gene to be inhibited is expressible in pathogenic organisms, preferably in plasmodia.

20. Use in accordance with one of the Claims 13 to 19, whereby the gene to be inhibited is a component of a virus or viroid.

21. Use in accordance with Claim 20, whereby the virus is a human pathogenic virus or viroid.

22. Use in accordance with Claim 20, whereby the virus or viroid is one that is pathogenic in animals or plants.

23. Use in accordance with one of the Claims 13 to 22, whereby the dsRNA is segmentally double-stranded.

24. Use in accordance with one of the Claims 13 to 23, whereby the ends of the dsRNA are modified to prevent degradation in the cell.

25. Use in accordance with one of the Claims 13 to 24, whereby the medicament is injectable into the bloodstream or into the interstitium of the organism being treated.

26. Use in accordance with one of the Claims 13 to 25, whereby the dsRNA and the vector that codes it, respectively, are taken up by bacteria or microorganisms.

Summary

The invention concerns a medicament containing at least one double-stranded ribonucleic acid (dsRNA) to inhibit the expression of a given gene, whereby one strand of the dsRNA is at least segmentally complementary to this gene.